Hydrodynamics and morphology of filamentous bacteria in a stirred tank fermenter

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ABSTRACT

Besides the positive effects of stirring (i.e. improvement of the mass, heat and momentum transfers), the induced turbulence may alter the microbial activity. Hydrodynamic stress is the generic term used to describe the unfavourable effects of the turbulence on the microorganisms. In order to contribute to a better knowledge of this complex phenomenon, we have studied the influence that mixing conditions may have on a filamentous bacterium : *Actinomadura R39*.

Investigations are carried out in a 5 litres batch fermenter operated at different stirring speeds and with two different types of impellers, known to produce different flow pattern within ther fermenter: Rushton turbines (radial flow) and Intermig (axial/radial flow).

The influence of hydrodynamics on *Actinomadura R39* is quantified by the evolution of its morphology, estimated by image analysis. The hydrodynamics in the fermenter is characterised using an artificial broth which has the same rheological properties than the fermentation broth, by the tracer methodology and power measurements. Tracer experiments are used to determine the mixing time and to represent the mixing flow pattern in the stirred tank by a compartmentalized model.

Relationships can be established between morphological modifications and hydrodynamic parameters characterizing the flow in the fermenter. Scale-up rules depend on the type of morphological parameter considered. The existence of such correlations between model parameters and the morphology also validates the physical signification of the compartimentalized model.

MATERIAL & METHODS

Strain: *Actinomadura* R39

Fermentation Broth :

– Starch	15 g/l
– Soy Pepton	12 g/l
– K_2HPO_4	2 g/l
– $MgSO_4.7H_2O$	0.6 g/l
– Trace elements	1 ml/l

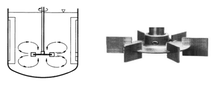
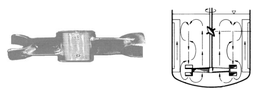
Artificial medium: (same rheological properties than the fermentation broth) non-newtonian viscous fluid :

– xanthan gum	1.25 g/l
– salts	2.6 g/l (see fermentation broth)
– antifoam	a few drops

Fermenter: Stirred tank reactor (B. Braun), 5 litres working volume

Gas: Air, 1 vvm (5 litres/min)

Impellers: (3 mobiles on the same shaft)

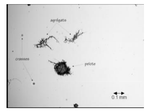
	Rushton Turbines	**Intermig**
		
Diameter:	0.056 m	0.102 m
Flow pattern:	radial	axial-radial

SYMBOLS

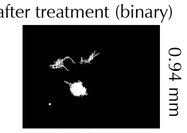
area	Pellets area (mm²)
d	Impeller diameter (m)
dFmax	Maximum Feret diameter (mm)
dFmin	Minimum Feret diameter (mm)
dz	Diameter of mobiles zone (m)
fp	Flow parameter (m³/s)
fpg	Flow parameter (with gas 5l/min (m³/s)
N	stirring speed (s^{-1})
Np	Power number (= $P/\rho N^3d^5$)
P	Power (W)
PC	Crofton perimeter (mm)
v_p	Peripheral tip speed (=πNd) (m/s)
θ_m	Mixing time (95%) (s)
ρ	Density (kg/m³)

IMAGE ANALYSIS

before treatment (grey scale picture)



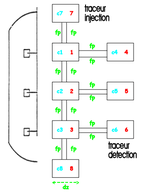
after treatment (binary)

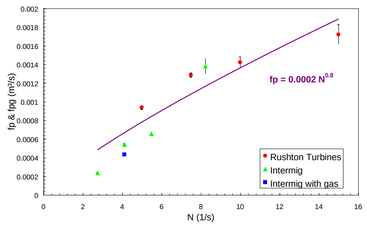


HYDRODYNAMICS

COMPARTIMENTALIZED MODEL

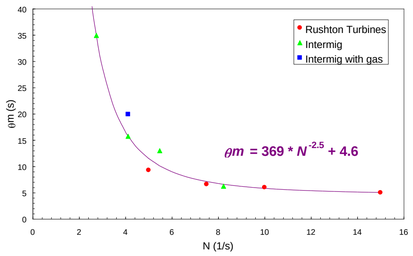
– 8 compartments
– 2 parameters : the pumping flow between the compartments *fp* (m³/s)
the diameter of the mobile zone *dz* (m)

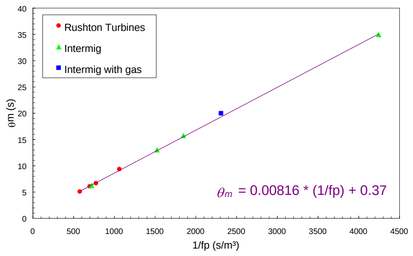




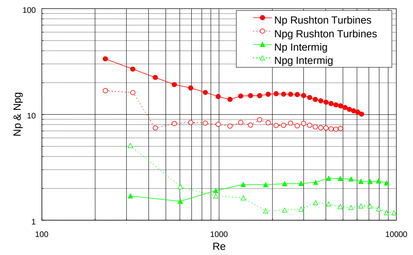
dz = constant : dz_{mean} = 0.073 m (± 0.008)

MIXING TIME



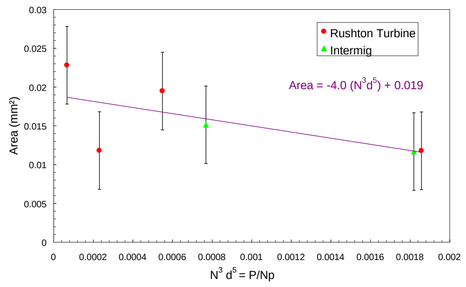


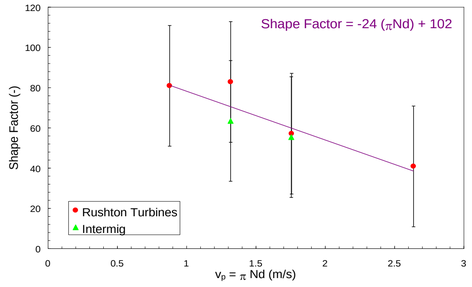
POWER

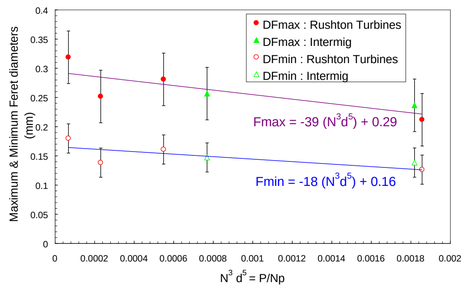


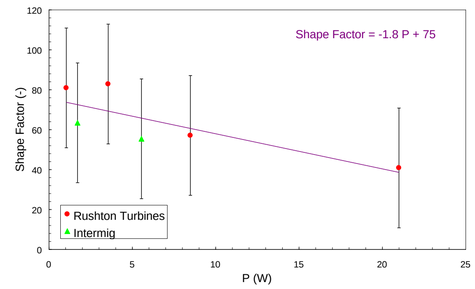
- **Rushton Turbines :** *Np* = 15.3 (± 0.3)
  Npg = 7.9 (± 0.4)
- **Intermig :** *Np* = 2.3 (± 0.1)
  Npg = 1.3 (± 0.1)

MORPHOLOGY ANALYSIS









Rap DF = DFmax/DFmin = 0.59 (±0.02)

Number of holes/mm² = 5465 holes/mm² (± 602)

$$Shape\ Factor = \frac{Area}{4\ \pi\ Perimeter^2}$$

CONCLUSIONS

The area and the Feret diameters (minimum and maximum) are correlated to N^3d^5, corresponding to the ratio *P*/*Np*, and to fp^3d^5. Those quantities are <u>global</u> characteristics of hydrodynamics of the fermenter. The relation to the ratio *P*/*Np* indicates that not only the power input is a important factor but also the way the impeller dissipates it into the bulk. Rushton turbines are high power number mobiles (*Np*=15.3) and Intermig are low power number impellers (*Np*=2.3). The shape factor is correlated to the impeller tip speed *Nd*, to the power input *P*, and to *fp d*. Those quantities are <u>local</u> characteristics of hydrodynamics of the fermenter (i.e. maximum shear stress). The ratio of the minimum and maximum Feret diameters is constant and independent of the cultivation condition. It is equal to 0.59. The density of holes (i.e. the number of holes per mm²) is also independent of the cultivation conditions. It is about 5500 holes/mm². Those correlations developed in this work are relevant for two different kind of impellers (Rushton turbines and Intermig) producing clearly different flow patterns

Beyond results related to a specific strain : *Actinomadura R39*,

this work proposes a general methodology to study the influence of mixing conditions on the morphology of filamentous bacteria. This morphology reflects the complex and multiscale influence of hydrodynamics: the microbial strain integrates during its growth spatial and temporal fluctuations of the hydrodynamic conditions encountered in the bulk of the fermenter. This work validates also the relevance of the physical signification of compartmentalized models to describe the mixing flow within a stirred tank fermenter.